

## SEQUENCE LISTING

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<120> MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD  
 OF SCREENING THERMOPHILIC BACTERIA USING THE SAME

<130> 04853.0048-00000

<140> 09/697,186  
 <141> 2000-10-27

<150> JP 309616/1999  
 <151> 1999-10-29

<160> 20

<170> PatentIn Ver. 2.1

<210> 1  
 <211> 253  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 1

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe  
 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser  
 100 105 110



Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 2

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 2

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110  
 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125  
 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140  
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160  
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175  
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu  
 180 185 190  
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205  
 Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220  
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240  
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 3  
 <211> 253  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 3  
 Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15  
 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30  
 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45  
 Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe  
 50 55 60  
 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp  
 85 90 95  
 Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110  
 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125  
 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140  
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160  
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175  
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu  
 180 185 190  
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Pro Gly Tyr Val Gln Leu  
 195 200 205  
 Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu  
 210 215 220  
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg  
 225 230 235 240  
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 4  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Primer for  
 PCR amplification

<400> 4  
 gactgtacgg gtacccgttg acggccgata tggta

35

<210> 5  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Primer for  
 PCR amplification

<400> 5  
 gactgtacgc tgcagcgtaa ccaacatgat taaca

35

<210> 6  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer for PCR amplification

<400> 6  
gactgtacgg aattcgagct cgagcaaatc taaaa 35

<210> 7  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer for subcloning of WT

<400> 7  
gactgtacgc atatgaatgg accaataata atgac 35

<210> 8  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer for subcloning of KT3-11 and HTK

<400> 8  
gactgtacgc atatgaaagg accaataata atgac 35

<210> 9  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Primer for subcloning

<400> 9  
gactgtacgc tcgagcgtaa ccaacatgat taaca 35

<210> 10  
<211> 759  
<212> DNA  
<213> *Staphylococcus aureus*

<220>  
<221> CDS  
<222> (1) .. (759)

tgc cag ttc gta atg tct ggt caa ctt tcc gac tct gag aaa ctt ctg	672
Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu	
210 215 220	
gaa tcg cta gag aat ttc tgg aat ggg att cag gag tgg aca gaa cga	720
Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg	
225 230 235 240	
cac gga tat ata gtg gat gtg tca aaa cgc ata cca ttt	759
His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe	
245 250	
<210> 11	
<211> 253	
<212> PRT	
<213> Staphylococcus aureus	
<400> 11	
Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val	
1 5 10 15	
His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys	
20 25 30	
Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr	
35 40 45	
Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe	
50 55 60	
Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp	
65 70 75 80	
Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp	
85 90 95	
Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser	
100 105 110	
Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala	
115 120 125	
Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe	
130 135 140	
Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr	
145 150 155 160	
Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu	
165 170 175	
Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu	
180 185 190	
Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu	
195 200 205	

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 12

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 12

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

Tyr Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe  
 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Leu Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 13

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 13

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asn Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe  
 50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Pro Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser  
 100 105 110

Gly Gly Tyr Leu Gly Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Leu Val Leu  
 180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 14

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 14

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser  
 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asn Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 15

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 15

Met Ser Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160  
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175  
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Thr Val Leu  
 180 185 190  
 Thr Glu Ala Val Lys Leu Ser Asp Leu Pro Ser Gly Tyr Asp His Leu  
 195 200 205  
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220  
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Thr Glu Arg  
 225 230 235 240  
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

&lt;210&gt; 16

&lt;211&gt; 253

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

&lt;400&gt; 16

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Thr Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp  
 85 90 95

Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Thr  
 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Leu Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Gly Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Val Arg Gln Pro Asp Leu Pro Pro Gly Tyr Asp His Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Ala Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

&lt;210&gt; 17

&lt;211&gt; 253

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

&lt;400&gt; 17

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 18

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 18

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp  
 85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110  
 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125  
 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140  
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160  
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175  
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu  
 180 185 190  
 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu  
 195 200 205  
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
 210 215 220  
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240  
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

&lt;210&gt; 19

&lt;211&gt; 253

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

&lt;400&gt; 19

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
 65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp  
 85 90 95

Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
 100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
 115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
 130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
 145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
 165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu  
 180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu  
 195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu  
 210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
 225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
 245 250

<210> 20

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Mutant enzyme  
 obtained by introduction of point mutation into  
 wild type KNT gene of *Staphylococcus aureus* and  
 its expression

<400> 20

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
 20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
 35 40 45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Glu Ala Glu Phe  
 50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Asp  
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp  
85 90 95

Pro Leu Thr His Gly Lys Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro  
100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu  
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu  
180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Val Gln Leu  
195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu  
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg  
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe  
245 250

end

31